MUHAMMAD HAZIQ SYAHIR BIN KHAIZURAN

MASTER OF SCIENCE

2017

ESTABLISHMENT OF TISSUE CULTURE TECHNIQUE TOWARDS TRANSFORMATION OF *ORYZA SATIVA* WITH *OMEGA-3 DESATURASE GENE*

MUHAMMAD HAZIQ SYAHIR BIN KHAIZURAN

MASTER OF SCIENCE
UNIVERSITI MALAYSIA TERENGGANU

2017

ESTABLISHMENT OF TISSUE CULTURE TECHNIQUE TOWARDS TRANSFORMATION OF *ORYZA SATIVA* WITH *OMEGA-3 DESATURASE GENE*

MUHAMMAD HAZIQ SYAHIR BIN KHAIZURAN

Thesis Submitted in Fulfillment of the Requirement for the Master of Science in the School of Fundamental Science Universiti Malaysia Terengganu

May 2017

CURRICULAR VITAE

PERSONAL DIRECTORY

NAME	:	Muhammad Haziq Syahir Bin Khaizuran
ADDRESS	:	12, Pesiaran Putra 9, Taman Putra Permai, Bandar Baru Putra, Bercham, 31400, Ipoh, Perak.
E-MAIL	:	haziqsyahir11@gmail.com
D.O.B	:	August, 18, 1991
RACE	:	Malay
RELIGION	:	Islam

ACADEMIC QUALIFICATION

2017	-	Universiti Malaysia Terengganu Master of Science (Biotechnology) – Expected October 2017
2013	-	Universiti Malaysia Terengganu Bachelor of Science (Biological Science)
2010	-	Perak Matriculation College
2008	-	Sekolah Menengah Kebangsaan Agama Slim River

EXTRA-CURRICULAR ACTIVITIES

2015	-	School of Science and Food Technology Seminar Day – Best Oral Presenter
	-	Malaysian Society of Plant Physiology Conference – Poster Presenter
2014	-	International Conference of Advances in Plant Biochemical and Biotechnology - Oral Presenter
2012	-	Terengganu Anti-Corruption Today Campaign - Head of Exhibition
	-	Biology Convention - Assistant Director of Exhibition
	-	Educational Trip to National University of Singapore (NUS)

Abstract of thesis presented to the senate of Universiti Malaysia Terengganu in fulfillment of the requirements for the degree of Master of Science

ESTABLISHMENT OF TISSUE CULTURE TECHNIQUE TOWARDS TRANSFORMATION OF *ORYZA SATIVA* WITH *OMEGA-3 DESATURASE GENE*

MUHAMMAD HAZIQ SYAHIR BIN KHAIZURAN

May 2017

Main Supervisor : Professor Aziz Bin Ahmad, PhD

School : School of Fundamental Science

Salinity stress in paddy field had retarded the annual rice productivity influenced by ionic imbalance, physiological damage and biochemical breakdown. Few reports had categorized rice as a salt sensitive crop manifested in slow vegetative growth rate and delayed reproductive stage. In parallel towards high rice market demands, the establishment of new rice variety with high tolerance towards salt is important to overcome the tragic rice agriculture lost due to soil problems. The opening of newly paddy arable land is impossible due to urbanization. Instead of an exploitation of plant genetic sources in conventional crop breeding, advances in molecular breeding should be priority due wider genetic materials. Omega-3-Fatty Acid Desaturase (*ω3-FAD*) gene from marine microalgae *Chorella Vulgaris* strain UMT-M1 was employed in this study as a desired insert as the gene clustered as a saline-combat regulator. An initiative in this new variety production require the optimization of powerful tissue culture protocol.

Results shows that B5 medium supplemented with 1 mg/L 2,4-D, 10 mg/L NAA, 1 mg/L casein hydrolysate, 2.5 mg/L L-proline, 5 % (w/w) maltose and 2 g/L phytagel produce the highest number of embryogenic calli in all MR219, MR263 and SS1-42 tested line. Histological observation confirmed the calli embryogenecity. During regeneration of calli somatic embryogenesis, only SS1-42 rice calli develop green photosynthetic spot in responses towards MS medium enriched with 1 mg/L IAA and 4 mg/L BAP. However, the calli was unable for further shoot and root formation. This low regeneration ability merged as the most critical parameter as mentioned by previous researchers. As an alternative, rice zygotic embryo and shoot plumule that rapidly obtained from sterilized seeds in free-hormone liquid MS medium was used as a gene-receiver explant. This candidate tissue does not shows tough regeneration as it continuously grows in free hormone MS medium. The addition of 3 mg/L BAP fasten the plant micropropagation progress while the implementation of half strength MS was important for rooting. The procedure for gene-transfer during plant-bacterial co-culture was modified to fix the nature of three (3) different tissue that is embryogenic calli, semi-embyrogenic zygotic embryo and non embryogenic shoot plumule. The manipulation designed was the type phenolic compound: vanillin and acetosyringone, concentration with and without heat shock treatment. Preliminary examination of vanillin as a phenolic compound on MR219 calli exhibit less efficient outcomes compared to acetosyringone at all tested 0, 100, 200 and 300 µM concentration. Meanwhile, the implementation of heat shock treatment had slightly elevated the transformation efficiency. Further experiment on all choosen explant shows same ascending pattern as the increment of acetosyringone concentration where the highest score was recorded in 300 µM with heat shock treatment. Moreover, hygromycin B (hyg B) antibiotic cytotoxicity on all explant type also was investigated to

determine the optimal hyg B concentration during transformant selection. Result showed that 50 µg/mL of hyg B was lethal dose for calli while 80 µg/mL in zygotic embryo and shoot. Putative transformant then was confirmed by *β-glucoronidase* (*GUS*) assay that gave blue spot coloration on the transgenic body. Green Fluorescene Protein (GFP) microscopy on transformed calli also has been observed as a simplest reporter gene analysis. Meanwhile, PCR analysis of putative transformant positively shows targeted DNA band, however, as the plant regenerated, some of the band disappeared validating that the insert does not fully inherited. Nevertheless, southern blot of insert segement conducted had proved that the inserted segment was fully integrated into the plant genome of calli and zygotic embryo tissue while transient in non-embryogenic sector. Remarking only pro-embryogenic tissue as the most suitable gene receiver explant. These transgenic inauguration factor was important in confronting the challenge creates by the rice recalcitrancy towards bacterium-assisted transformation. The result obtained from this research can be applied in future crop transformation with an effective cost.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

PENGHASILAN TEKNIK KULTUR TISU KE ARAH TRANSFORMASI *ORYZA SATIVA* DENGAN *GEN OMEGA-3 DESATURASE*

MUHAMMAD HAZIQ SYAHIR BIN KHAIZURAN

Mei 2017

Penyelia Utama : Profesor Aziz Bin Ahmad, PhD

Pusat Pengajian : Pusat Pengajian Sains Asas

Masalah kemasinan tanah di kawasan tanaman padi telah merencatkan produktiviti tahunan padi dipengaruhi oleh ketidakseimbangan ionik, masalah fisiologi dan keterenjatan proses biokimia. Beberapa laporan telah mengesahkan padi sebagai tanaman sensitif garam yang melambatkan kadar pertumbuhan vegetatif dan pembiakan. Dalam usaha memenuhi permintaan pasaran yang tinggi, penghasilan varieti padi baharu yang mempunyai daya tahan kemasinan yang tinggi amatlah penting. Pembukaan kawasan tanaman padi yang baharu adalah seakan mustahil kerana masalahn kekurangan tanah. Selain daripada kebergantungan kepada kaedah pembakaan konvensional melualui sumber genetik tumbuhan, kemajuan dalam bidang pembakaan pada peringkat molekular seharusnya diberikan perhatian kerana sumber genetik yang lebih luas skopnya. Dalam kajian ini, gen Omega-3-Penyahtepu Asid lemak (*ω3-FAD*) telah digunakan kerana ianya diklasifikasikan sebagai pengawal regulatasi masalah kemasinan. Inisiatif dalam menghasilkan varieti baharu ini memerlukan protokol tisu kultur yang terbukti berkesan.

Hasil kajian mendapati media B5 yang ditambah 1 mg/L 2,4-D, 10 mg/L NAA, 1 mg/L asid kasien hidrolis, 2.5 mg/L L-prolin, 5 % (w/v) maltosa dan 2 g/L gel *'phyta'* menghasilkan kalus embriogenik dalam kesemua MR219, MR263 dan SS1-42 varieti yang diuji. Pengesahan status embriogenik kalus telah dijalankan melalui pemerhatian histologi. Untuk eksperimen regenerasi pokok melalui embriogenesis sel soma, hanya kalus SS1-42 mempunyai tindak balas positif melalui pertumbuhan bintik hijau yang mencirikan pigmentasi fotosintesis awal. Media yang digunakan adalah MS diperkaya 1 mg/L IAA dan 4 mg/L BAP. Walaubagaimanapun, kalus tersebut gagal menghasilkan batang mahupun akar untuk regenerasi yang seterusnya. Kekurangan daya regenerasi semula itu kekal sebagai masalah utama seperti yang telah dilaporkan oleh penyeledik sebelum ini. Sebagai alternatif, embrio zigot dan batang plumul telah pun digunakan sebagai tisu penerima gen asing. Kedua-dua tisu ini didapati daripada biji benih yang dikultur dalam media MS cair tanpa hormon. Tisu ini juga tidak mempunyai masalah regenerasi semula. Proses mikropropagasi dapat dicepatkan dengan menambahkan 3 mg/L BAP diikuti penggunaan MS separuh nutrien untuk menggalakkan pertumbuhan akar. Prosedur penghantaran gen melalui proses kultur bersama antara eksplan dan bakteria telah diubah sedikit di dalam kajian ini untuk memastikan ianya praktikal kepada tiga (3) jenis tisu eksplan iaitu kalus embriogenik, semi-embriogenik zigot, dan sektor tidak embriogenik plumul batang. Manipulasi yang dijalankan adalah jenis sebatian fenolik: vanilin dan asetosyringon, kepekatan dan kesan renjatan haba. Dapatan awal ke atas kalus MR219 mendapati vanilin menunjukkan kesan yang kurang efisen jika dibandingkan kepada asetosyringon pada semua kepekatan yang dicuba iaitu 0, 100, 200 dan 300 µM. Manakala pelakuan renjatan haba berjaya meningkatkan sedikit kadar transformasi tanpa signifikan yang ketara. Eksperimen selanjutnya kepada semua eksplan

menunjukkan corak peningkatan yang sama selaras dengan kenaikan kepekatan asetosyringon. Kesemua eksplan menunjukkan pencapaian yang tertinggi pada 300 µM berserta rawatan renjatan haba. Selain itu, ujian sitotoksik antibiotik hygromin B terhadap kesemua jenis eksplan juga telah dilakukan untuk mengenalpasti kepekatan optimum yang sepatutnya digunakan semasa sesi saringan transforman. Keputusan mendapati 50 µg/mL adalah sudah cukup untuk menjadi dos letal kepada kalus manakala 80 µg/mL kepada plumul batang. Penelitian *β-glukoronidase* (*GUS*) yang menghasilkan warna biru biru pada individu transgenik dan ujian mikroskopi 'Green Fluorescene Protein (GFP)' juga telah dijalankan sebagai analisisa gene penanda yang paling mudah. Analisa awal PCR menunjukkan kehadiran DNA yang disisip masuk kedalam eksplant, bagaimanapun, apabila eksplant dijana semula, beberapa kehilangan DNA telah dikenalpasti sekaligus mengesahkan bahawa sisip yang dimasukkan tidak semuanya diwariskan secara menyeluruh. Namun, Analisis pertindihan 'southern' yang dilakukan mengesahkan bahawa sisip yang dikendaki tersebut hanya berintegrasi di dalam genom kalus dan embrio zigot manakala bersifat peralihan-sementara dalam sektor tidak embriogenik. Keputusan ini menunjukkan bahawa hanya pro-embriogenik tisu mempunyai potensi sebagai eksplan penerima gen asing. Kesemua hasil dapatan kajian sebaiknya diaplikasikan dalam transformasi tumbuhan tanaman di masa akan datang dengan kos yang efektif kerana maklumat yang tercatat di dalam laporan ini telahpun serba sedikit dapat mengatasi masalah kalis tanaman padi kepada transformasi menggunakan bakteria.